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نموذج رقم (18)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية
وقوانين الجامعة الأردنية وأنظمتها وتعليماتها
لطلبة الماجستير

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عنوان الرسالة: Growth interaction between lactic acid bacteria starter culture with or without Bifidobacterium spp. on the growth of Staphylococcus aureus during milk fermentation and storage.

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير عندما قمت شخصيا" باعداد رسالتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الرسائل العلمية. كما أنني أعلن بأن رسالتي هذه غير منقولة أو مستلة من رسائل أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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GROWTH INTERACTION BETWEEN LACTIC ACID BACTERIA STARTER CULTURE WITH OR WITHOUT *Bifidobacterium spp*. ON THE GROWTH OF *Staphylococcus aureus* DURING MILK FERMENTATION AND STORAGE.

By

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Supervisor

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This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master's Degree of Science in Food Science and Nutrition

Faculty of Graduate Studies

The University of Jordan

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This Thesis (Growth interaction between lactic acid bacteria starter culture with or without *Bifidobacterium spp*. on the growth of *Staphylococcus aureus* during milk fermentation and storage) was successfully defended and approved on April 20th, 2011.

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DEDICATION

To *my Parents who lightened my way*

To *my wife and brothers who always support me*

To *my spiritual father who guide me supervisor Prof. Khalaf S. AL-Delaimy*

To *my company manager who support me through my work and study Mr. Walid finan*

To *my spiritual brothers who support me always Mr. Younes Al-ayaseh and Mr. Osama Al-Khatib*

To *all of my supportive colleagues and friends*

To all of you, I dedicate this effort……

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Yaser Hamamdeh

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BHI	Brain Heart Infusion Broth
BPA	Baird Parker Agar
LAB	Lactic Acid Bacteria
MRS	de Man, Rogosa and Sharpe Agar

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GROWTH INTERACTION BETWEEN LACTIC ACID BACTERIA STARTER CULTURE WITH OR WITHOUT *Bifidobacterium spp*. ON THE GROWTH OF *Staphylococcus aureus* DURING MILK FERMENTATION AND STORAGE.

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ABSTRACT

This study was designed to find out the growth interaction between the enterotoxigenic bacteria *Staphylococcus aureus* and yoghurt lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (LAB) and the probiotic bacteria *Bifidobacterium lactis* during milk fermentation to yoghurt at 45° C for 4 hour and storage for 12 days at room (20-25° C) and refrigeration (4° C) temperatures. Growth interaction between *S. aureus* and LAB resulted in a significant decrease at ($P < 0.01$) (1.2 log_{10} / ml after 6 days with complete inhibition after 9 days and 1.61 log_{10} / ml after 9 days with complete inhibition after 12 days) in *S. aureus* counts during milk fermentation and storage at room and refrigeration temperatures, respectively. The counts of LAB were significantly increased during the same period of fermentation and storage at room temperature. The decrease in *S. aureus* count and increased of LAB counts were relatively less in refrigeration than at room temperatures. The growth interaction between *S. aureus* and *B. lactis* was generally similar to that between *S. aureus* and LAB. The counts of *S. aureus* were 2.9 log_{10} after 3 days with complete inhibition after 6 days and 2.63 log_{10} after 6 days with complete inhibition after 9 days at room and refrigerated storage respectively. The growth interaction between *S. aureus* and combined LAB and *B. lactis* showed a higher inhibition of *S. aureus* (1.76 log_{10} after 3 days with complete inhibition after 6 days and 1.05 log_{10} after 6 days with complete inhibition after 9 days, for room and refrigerated storage, respectively) than the inhibition by any of LAB or *B. lactis* alone. Increasing the initial numbers of each bacterium to approximately one log did not significantly affect the growth interaction between these bacteria. The pH values during milk fermentation and storage were significantly at ($P < 0.01$) reduced.

The enterotoxigenic bacteria *S. aureus* was completely inhibited by the growth of LAB and *B. lactis* separately and the combination of the two during milk fermentation and storage at both room and refrigerated temperatures. Therefore it may be possible to

recommend the use of *B. lactis* (as probiotic) with LAB in milk fermentation to yoghurt and storage.

1. Introduction:

Staphylococcal food poisoning is one of the most common types of food borne disease worldwide. It is intoxication, resulting from the ingestion of food containing one or more performed *Staphylococcal* enterotoxins (Cliver and Riemann, 2002). Among *Staphylococci* species *S. aureus* is the most important species that causes food borne diseases. *S. aureus* is pathogenic Gram positive bacterium and the causative agent of a wide panel of infections ranging from superficial lesions to life threatening septicemia. The natural ecological niches of the species are the nasal cavity and skin of the warm blooded animals. Because of its importance and highly prevalence, there is an increasing interest in bacterial interactions and in understanding of the mechanisms by which the inhibition of *S. aureus* by other bacterial species may occur (Charlier, et al. 2009).

Lactic acid bacteria (LAB) have been used in food fermentation for centuries. LAB plays an important role in food industry, because they contribute to the flavor, texture, and nutritional values of the food products. The interest in application of LAB and their metabolites in food industry mainly related to the prevention of food spoilage and extending of shelf life of foods, and the capacity to inhibit the growth of pathogenic microorganisms (Topisirovic, et al., 2006). The inhibition of *S. aureus* by LAB has been widely studied. Abee et al., (1995) reported that LAB, as starter culture are routinely employed in the fermented food and food products, to inhibit the growth of spoilage and pathogenic bacteria in fermented meat, dairy and other groups of food products.

Bifidobateria are natural component of the gastrointestinal microbiota, and are generally recognized as probiotics "live microorganisms which when administered in adequate

amounts confer a health benefit to the host" (Martins, et al., 2010). Several health promoting properties of *Bifidobacterium* have been proposed, including inhibition of pathogenic bacteria in the intestine. One of the proposed mechanisms of inhibition is through production of antimicrobial substances. Antimicrobial components produced by *Bifidobacterium* include organic acids, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins (Lahtinen, et al., 2007).

No available studies (as far as we know) are found on the growth interaction between *Bifidobacterium* as probiotic, with *S. aureus* as pathogenic food contaminant affecting human health, with or without the presence of LAB starter culture, during milk fermentation process and storage.

Therefore this study focused on studying the growth interaction between the enterotoxigenic bacteria *S. aureus* with LAB starter culture and *B. lactis* during milk fermentation to yogurt and during storage, as it's of importance from the health and safety point of views.

2. Literature Review:

Staphylococcus was first recognized as a genus on 1884 and belongs to the family *Micrococcaceae*. The *Staphylococci* are Gram positive cocci, 0.5-1.5µm in diameter and can occur as single cells, in pairs, or as cultures (Cliver and Riemann, 2002).

The *Staphylococcal* food poisoning or food intoxication syndrome was first studied in 1894 by J. Denys and later in 1914 by Barber, who produced in himself the signs and symptoms of the disease by consuming milk that had been contaminated with a culture of *Staphylococci*. (Jay, 2000).

Staphylococcal food poisoning is caused by the ingestion of enterotoxins produced by the *Staphylococci* in the food. *Staphylococci* have to grow in the food to produce enterotoxins; however not all *Staphylococci* are enterotoxigenic. Nine serological distinct enterotoxins have been identified based on their reactions with specific antibodies; they are designated enterotoxin A (SEA), B (SEB), C (SEC), D (SED), E (SEE), G (SEG), H (SEH), I (SEI) and J (SEJ). SEA is the enterotoxin most frequently associated with *Staphylococcal* food borne outbreaks. The presence of large number of *Staphylococci* in the food may indicate poor handling or sanitation. On the other hand, absent of *Staphylococci* in the food does not necessarily indicate that enterotoxin is absent because the organism are easily destroyed by heat, whereas the entertoxins are not (Cliver and Riemann, 2002).

While a range of *Staphylococcus* species can produce enterotoxins, the majority of outbreaks of *Staphylococcus* food poisoning are due to the presence of *S. aureus* (Bremer, et al., 2004). *S. aureus* is a pathogenic, non motile, spherical, gram positive bacterium.

Certain *S. aureus* strains can produce enterotoxins and cause *Staphylococcal* food poisoning (Charlier, et al., 2009).

S. aureus has been recognized as an indicator of deficient hygiene of food and processing and a major cause of food gastroenteritis worldwide. An enterotoxin dose of ≤ 1 µg in contaminated food produces symptoms of *Staphylococcal* intoxication, but this toxin level is typically reached only when *S. aureus* populations exceed 5 $\log_{10}$ cfu/ g. If environmental conditions during food storage and preparation allow the growth of *S. aureus* (i.e. time and temperature abuse) *Staphylococcal* entertoxins may produced, being potentially harmful for consumers (Valero, et al., 2009). European legislation stipulates the obligation of testing for the presence of enterotoxins in dairy products if *S. aureus* is detected at concentrations over 5 $\log_{10}$ cfu/ g (Le Marc, et al., 2009), (Paus, et al., 2010).

S. aureus is often found closely associated with human body. It may also be found in many parts of our environment, including dust, water and air and on clothing, utensils and work surfaces. Although *S. aureus* is an important pathogen, many healthy people carry it as of the normal population of micro-organisms associated with nose, throat, perineum or skin. The carrier rate varies in different populations. The nasal passages are reported to harbor *S. aureus* in 10-50% of the healthy population. *S. aureus* causes several infections that compromise food safety because of their frequency and the fact that they do not necessarily prevent the infected person from working. Various types of skin eruptions and inflammations and wounds, sometimes as small minor damage around fingernails, can harbor large numbers of the organisms. *S. aureus* can also cause respiratory infections or may become established in the gut, causing enteritis (Bremer, et al., 2004).

The symptoms of *Staphylococcal* food poisoning usually develop within 4 hour of the ingestion of contaminated food, although a range of 1-6 hour has been reported. The symptoms include nausea, vomiting, abdominal cramps, diarrhea, sweating, headache, prostration, and sometimes a fall in the body temperature, generally last from 24-48 hour (Jay, 2000).

S. aureus growth and enterotoxin production are influenced by a variety of environmental and nutritional factors including temperature, pH, water activity, inoculums size, atmospheric composition, carbon and nitrogen sources, salt level, and competing micro flora. *S. aureus* is capable to grow in a wide range of temperatures, ranging from 5 to 48.5º C with an optimum of 30º to 37º C; pH between 4.0 to 10.0 with an optimum of 6.0 – 7.0; and sodium chloride concentrations up to 25 %. These characteristics enable *S. aureus* to grow and survive in a wide range of environmental conditions as well as to persist in stressful environment (e.g. dry surfaces) for long periods (Valero, et al., 2009). Enterotoxin is produced by *S. aureus* at 10-40º C with an optimum range of 37-40º C, pH value between 5.15-9.0, and sodium chloride concentrations up to 10 % (Cliver and Riemann, 2002).

Fujikawa and Morozumi. (2006) reported that at constant temperatures from 14-36.5º C, the amount of toxin in milk increased linearly with time from the time the cell population reached 6.5 $\log_{10}$ cfu/ ml.

Bremer et al. (2004) reported that foods that are frequently implicated in *Staphylococcal* food poisoning include meat and meat products, poultry and egg products, and salads such as egg, tuna, chicken, potato, and macaroni. Also implicated are bakery products such as

cream filled pastries, cream pies, chocolate éclairs, sandwich fillings, and milk and dairy products. Foods that require considerable handling during preparation and that kept at slightly elevated temperatures after preparation are frequently involved in *Staphylococcal* food poisoning. Oulahal, et al. (2008) also reported that *S. aureus* is sometimes associated with raw milk and milk products as a result of bio-films formed on the surfaces of milk processing equipment. Rodriguez, et al. (2005) reported that *S. aureus* is a pathogen of major concern for the dairy industry.

Le Marc et al. (2009) reported that *S. aureus* has been responsible for several outbreaks involving dairy products, in June and July 2000, more than 14700 people become ill after consuming dry skim milk powder contaminated with *Staphylococcal* enterotoxin A. Thirty five outbreaks associated to *S. aureus* toxins were reported in the European Union in 2006, which resulted in 777 cases, 14 hospitalizations and 1 death. In Spain the number of cases reported increased from 442 cases in 2001 to 649 cases in 2006 (Valero, et al., 2009).

An extensive *S. aureus* food poisoning outbreak among patients who ingested dairy products occurred in Osaka, Japan, in 2003. The cause was *Staphylococcal* enterotoxin A (SEA) contamination of the dairy products as a result of abuse temperature during processing (Fujikawa and Morozumi, 2006).

LAB compose of a heterogeneous bacterial group comprising non-sporulating, gram positive *cocci* and *bacilli*, whose principle common characteristic is the ability to ferment sugars predominantly into lactic acid which in turn leads to an acidification of the environment down to a pH 3.5. These bacteria have facultative anaerobic metabolism and do not produce catalase (Charlier, et al., 2009). LAB refers to species of the following

genera: *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* (Reuter, 1985). LAB are generally mesophilic but can grow at temperatures as low as 5º C or as high as 45º C. similarly, while the majority of strains grow at pH 4.0-4.5, some are active at pH 9.6 and others at pH 3.2. Strains are generally weakly proteolytic and lipolytic and require preformed amino acids, purine and pyrimidine bases and B vitamins for growth (Caplice and Fitzgerald, 1999). Kluyver divided LAB into two groups based on the end products of glucose metabolism. Those that produce lactic acid as the major or sole product of glucose fermentation are designated homo-fermentative, and those that produce equal molar amounts of lactate, carbon dioxide, and ethanol from hexoses are designated hetero-fermentative (Jay, 2000).

LAB play an important role in food preparation and preservation, where lactic fermentation is one of the oldest forms of preparation and preservation of foods such as cheese, yogurt, fermented milk and butter, sourdough breads, curing, sauerkraut as well as vegetable brining and silage. LAB also contributes to the development of the organoleptic characteristics (flavor, aroma, and texture) of the final products. Their ability to promote food preservation is linked to the fact that they cause a decrease in pH, as a consequence of lactic acid production, and additionally, the production of a number of antimicrobial agents, a combination of these factors limits the proliferation of undesirable microorganisms (spoilage or pathogenic). LAB therefore plays a role in promoting food safety (Charlier, et al., 2009).

The genus *Lactobacillus* is the largest group among LAB containing, at present more than 120 species and 20 subspecies. The *Lactobacilli* are Gram positive, non spore-forming microorganisms. Considering cellular shape, they can occur as rods or coccobacilli, and are

included in the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family *Lactobacillaaeae*. They are strictly fermentative (either homo or heterofermenters), aerotolerant or anaerobic, aciduric or acidophilic having complex nutritional requirements (carbohydrates, amino acids, peptides, fatty acid esters, salts, vitamins). They are naturally associated with a large variety of nutritive rich plant and animal derived environments, and many species are involved in the manufacture and preservation of fermented foods (such as milk, meat, vegetables, and cereals) in which they are present as contaminants. *Lactobacilli* are naturally inhabitants of the gastrointestinal tracts of human and animals, where they are thought to play pivotal roles in maintenance and recovery of a healthy state. A number of *Lactobacilli* strains have been used as probiotics. Beneficial effects attributed to indigenous and probiotic *Lactobacilli* include colonization of intestinal mucosa, inhibition of pathogens, immuno modulation, and cholesterol assimilation (Lee and Salminen, 2009).

The genus *Streptococcus* contains about 60 species. They are Gram positive, catalase negative, non-motile, non-spore forming and homo-fermentative microorganisms, and are included in the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family *Streptococcaceae* (Felies and Dellaglio, 2010).

In the dairy industry, starters of mixed cultures of LAB are used among which the well known yoghurt culture, mixture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophillus*. Regarding viability, the norm specifies that the sum of microorganisms constituting the starter culture should be at least 7 log_{10} cfu/ g (Camaschella, et al., 1998).

Tissier was first to described *Bifidobacteria* in 1900. He noted that *Bifidobacteria* were the dominant bacteria in the stools of breast fed infants and were numerically significant than in stools of bottle fed infants (Ishibashi, et al., 1997). *Bifidobacteria* are dominant normal microbiota of human gastrointestinal tract where they represent up to 25% of the cultivable fecal bacteria in adults and 80% in infants. Following normal delivery, these bacteria colonize the neonatal intestine from the first week after birth and inhabit the gastrointestinal tract throughout life, where they contribute to human health and well-being (Martins, et al., 2010). Microorganisms of the genus *Bifidobacterium* are non spore forming, non motile and non filamentous rods, which display various shapes, with slightly bifurcated club shaped or spatulated extremities. They can be found singularly, in chains, in aggregates, in V or palisade arrangements. They are strictly anaerobic, although some species can tolerate low oxygen concentrations, and they have fermentative metabolism. They were first included among the family *Lactobacillaaeae*, but in 1924 Orla-Jensen proposed the reclassification of the species *Lactobacillus bifidum* into new genus *Bifidobacterium* (Lee and Salminen, 2009). The genus *bifidobacterium* contains about 29 species, and are included in the phylum *Acintobacteria*, class *Acintobacteria*, subclass *Acentobacteridae*, order *Bifidobacteriales*, family *Bifidobacteriaceae* (Felies and Dellaglio, 2010). The optimum growth temperature for *Bifidobacteria* is 37-41º C, and the optimum pH is 6.5-7.0 (Roy, 2001). Over the past two decades, there has been a worldwide increase in the consumption of dairy products containing probiotic bacteria. In order to exert positive health effects, the microorganisms need to be viable, active and abundant the concentration of at least 6 log_{10} cfu/ gm in the product throughout the specified shelf life (Ostlie, et al., 2003).

The activities of one of bacterial species may be influenced by the growth activities of others. Bacterial antagonism could arise from combined effects of several mechanisms during their growth in the media. For example, one group of microorganisms may remove a growth factor required for the growth of another, synthesize a substance inhibitory to another or produce an adverse pH (Al-Delaimy and Kanakri, 2008).

LAB strains were reported to exert a strong inhibitory effect on *S. aureus* growth in milk. Several suggestions have been proposed for inhibition of *S. aureus* by LAB. These include production of bacteriocins (Bacteriocins are ribosomally synthesized peptides or proteins secreted by certain strains of bacteria), hydrogen peroxide, and organic acids such as lactic and acetic acids, and competition for nutrients (Wysocki, et al., 2010), (Cabo, et al., 2002), (Sameshema, et al., 1998). Le Marc et al. (2009) reported that the inhibition of pathogens including *S. aureus* by LAB is usually attributed to the production of weak acids, especially lactic acid. During fermentation process, the lactic acid produced decreases the pH by the process of dissociation. Both decreasing pH and increasing un-dissociated lactic acid have inhibitory effects on any pathogenic bacteria possibly present. Fandos et al., (1994) showed that starter culture with great acidification ability have a strange antagonistic effect against *S. aureus*, the factor mainly responsible for inhibition of *S. aureus* growth seems to be a rapid pH decrease. Wysocki et al. (2010) reported that antimicrobial effect produced by LAB appears to be unrelated to the acidification of the medium. Charlier et al. (2008) showed that a significant inhibition of *S. aureus* growth was observed in milk medium both when the pH was regulated or not, suggesting that acidification is not involved in the early inhibition of *S. aureus* growth by LAB in milk. Alomar et al. (2008) and Stecchini et al. (1991) reported that the inhibitory effect of the

LAB starter culture on *S. aureus* growth was not only due to the decrease in pH. The inhibition could be due to the production of antimicrobial substances. Charlier et al. (2009) reported that the antagonistic potential of LAB may also involve the production of bacteriocins which are active against *S. aureus*. It was reported that typical LAB bacteriocins have a narrow antimicrobial spectrum, but also LAB produces bacteriocins having broad antimicrobial spectrum. Thus, some LAB bateriocins can inhibit the growth of pathogenic and spoilage bacteria including *S. aureus* (Topisirovic, et al., 2006). LAB bacteriocins have a common mechanism of action by the formation of transitory portion complexes or ionic channels in the cytoplasmic membrane that causes total or major dissipation of the proton motive force (Arques, et al., 2011). Le Marc et al. (2009) reported that the cessation of *S. aureus* growth is due to accumulation of anti *Staphylococcal* substances produced by LAB. It was reported that the production of hydrogen peroxide by LAB particularly by *Lactobacilli*, is also antagonistic to *S. aureus* (Otero and Macias, 2006). Some *Lactobacilli* strains are able to inhibit the growth of *S. aureus* by producing hydrogen peroxide at concentration of 0.18 mmol/ Liter, hydrogen peroxide has a bacteriostatic effect at these concentration and bactericidal for concentration up to 0.6 to 1.0 mmol/ L (Charlier, et al., 2009). Gilliland and Speck (1974) showed that the inhibition of *S. aureus* in milk during mixed cultures with *Lactobacilli* and *Streptococci* strains was independent of hydrogen peroxide production. Ito et al. (2003) screened LAB from various food samples and evaluated their hydrogen peroxide production and he only observed a weak effect on *S. aureus* growth.

3. Materials and Methods:

3.1. Materials:

3.1.1. Milk samples:

Fresh pasteurized cow's milk samples used in these experiments were obtained from The University of Jordan dairy plant. Characteristic of milk are as follows (Fat content; 3.65%, Lactose content; 3.63%, Total solid; 1.04%, Protein content; 2.55%, Density; 28.31).

3.1.2. Bacterial strains:

B. lactis pure certified strain (HOWARU) as a probiotic and milk starter culture *L. bulgaricus* and *S. thermophillus* (YO-MIX 883) were supplied by DANISCO local representative. Bacterial strains were stored and reactivated according to the manufacturer instruction (Appendix A)

enterotoxigenic *S. aureus* wild strain was isolated from different food samples, purified, characterized, and tested for its ability to produce enterotoxin. Bacterial strain was kept on tryptone soya slant at 5º C until used in the experiments (Le Marc, et at., 2009).

3.1.3. Media:

All media were prepared according to the manufacturer instruction then sterilized in the autoclave at temperature of 121º C for 15 minutes (Appendix B).

3.2.: Methods:

3.2.1.: Isolation and characterization of *S. aureus*:

Wild enterotoxigenic *S. aureus* isolates were isolated from different food samples (white cheese, cow's milk, chicken, lamb and beef meat) purchased from local market according to the AOAC official method 987.09 as follows:

Fifty gm was weight from each food sample, then added to 450 ml sterilized peptone water and homogenize in a stomacher for two minutes at high speed. This 1:10 dilution used to prepare further serial dilutions up to approximately 10^{-6}. From each dilution 1 ml was inoculated into tube containing sterilized trypticase soya broth with 10 % sodium chloride and 1 % sodium pyruvate, and then incubated for 48 hour at 35-37º C. From each growth positive tube one loopful was transferred and streaked to Baird Parker agar (BPA) plates enriched with egg yolk tellurite emulsion (50 ml emulsion/ Liter BPA) to obtain isolated colonies. BPA plates was Incubated for 48 hours at 35-37º C.

Tests to confirm the ability of *S. aureus* isolates to produce enterotoxins were done according to the procedures of Kandler and Weiss (Bergey's manual, 1986), Bridson (Oxoid manual, 2006) and Cunniff (AOAC ,1995) include coagulase, catalase, and DNase and as follow:

a. Coagulase test:

One suspected typical *S. aureus* colony was transferred into a tube containing 0.2 ml brain heart infusion (BHI) broth, and then Incubated for 18-24 hour at 35-37º C, 0.5 ml reconstituted rabbit plasma then was added and mixed thoroughly, then Incubated at 35-37º C and examined periodically over 6 hour interval for clot formation (Any degree of clot formation is considered positive reaction).

b. Catalase test:

One suspected typical *S. aureus* colony was transferred into slide containing 0.5 ml hydrogen peroxide solution 3% then watched for formation of air bubbles as positive reaction.

c. DNase test:

One suspected typical *S. aureus* colony was streaked over DNASE agar plates, and incubated for 18-24 hours at 35-37º C, then flooded with 1 N HCL and allow them to stand for few minutes (where polymerized DNA precipitates in the presence of 1 N HCL and make the medium opaque). Observe if the suspected *S. aureus* isolate produce DNase enzymes that hydrolyze the DNA present in the medium. Clear zones are seen surrounding the colonies which indicate positive reaction (Bridson, Oxoid manual, 2006).

3.2.2.: Determination of *S. aureus* cell count:

One colony of the isolated enterotoxigenic *S. aureus* were transferred into tube containing 5 ml sterilized BHI broth, and Incubated for 24 hour at 35-37º C, then the cell count of growth positive tube then was enumerated according to AOAC official method 975.55 as follows:

One ml of growth positive tube was transferred into a tube containing 9 ml sterilized peptone water, this 1:10 dilution was used to prepare further serial dilutions up to 10^{-9}. One ml of each dilution then was aseptically transferred into triplicate plates of BPA, and equitably distributed over the triplicate plates (0.4 ml, 0.3 ml, and 0.3 ml), and spread over surface of agar using sterile, bent glass streaking rods, plates then Retain in upright position until inoculums is absorbed by medium, plates then inverted and incubated for 45-48 hour

at 35-37º C. Add number of colonies on appropriate triplicate plates and multiply by dilution factor to get the cell count of *S. aureus* / ml BHI broth.

3.2.3.: Growth experiments of bacteria in milk:

All experiments were done in duplicate, Gram stain was used to confirm the identity of each bacteria grown in the enumeration medium.

a. *S. aureus* growth in milk:

Approximately 6 log_{10} viable cells of enterotoxigenic *S. aureus* isolate / ml (obtained by inoculating one ml of BHI broth which has been inoculated with one colony of enterotoxigenic *S. aureus* isolate and incubated for 24 hour at 35-37° C) was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (*S. aureus* count was monitored initially then after 4 hour; end of incubation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). *S. aureus* count was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days and 12 days from the time of inoculation. *S. aureus* was enumerated according to Cunniff AOAC official method 975.55 as follows:

One ml of appropriate dilution of each sample was aseptically transferred into triplicate plates of BPA, and equitably distributed over the triplicate plates (0.4 ml, 0.3 ml, and 0.3 ml). Spread inoculums over surface of agar using sterile, bent glass streaking rods; avoid extreme edges of the plate. Retain plates in upright position until inoculums is absorbed by medium. Invert plates and incubate for 45-48 hour at 35-37º C. Add number of colonies on

appropriate triplicate plates and multiply by dilution factor to get the cell count of *S. aureus* / gm of test sample.

b. *B. lactis* growth in milk:

Approximately 6 $\log_{10}$ viable cells of *B. lactis* / ml (obtained by taking one ml of 10^{-9} serial dilution of *B. lactis* culture powder; each gram contain approximately 11 $\log_{10}$ viable cells) was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (*B. lactis* count was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). *B. lactis* count was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days and 12 days from the time of inoculation. *B. lactis* was enumerated by aseptically transferring 0.1 ml of appropriate dilution to each of duplicate MRS agar plates, supplemented with 1% raffinose as source of energy, 0.05% lithium chloride to suppress *Lactobacilli* (*L. bulgaricus*) growth, 0.05% L-cycteine hydrochloride which helps in providing anaerobic condition for *B. lactis* growth, then inoculums was spread over surface of agar using sterile bent glass streaking rods avoiding extreme edges of the plate, then Retain plates in upright position until inoculums is absorbed by medium and then plates was inverted and incubated in anaerobic jars for 72 hour at 45º C. the incubation temperature suppresses the growth *S. theromphilus*. Therefore the medium is selective for *B. lactis* (Tabasco, et al., 2007). Calculate the average of colonies in duplicate plates of

appropriate dilution and multiply by dilution factor and ten (cell count = average of colonies x dilution factor x 10). To get the cell count of *B. lactis* / gm of test sample.

c. Yoghurt lactic acid bacteria growth in milk:

Approximately 6 $\log_{10}$ viable cells of yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) / ml (obtained by adding two botch of mixture culture of LAB to 500 liter pasteurized milk) was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (LAB count was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). LAB count was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days and 12 days from the time of inoculation. *S. thermophilus* was enumerated by aseptically transferring 0.1 ml of appropriate dilution to each of duplicate M17 agar plates, supplemented with 19 gm / Liter di-sodium glycerophosphate which has sufficient buffering capacity to maintain the pH above 5.7 which enhance the growth of *S. thermophilus* and suppress the growth of *L. bulgaricus* (Bridson, Oxoid manual, 2006), inoculums then was spread over surface of agar using sterile bent glass streaking rods, avoiding extreme edges of the plate, plates then retained in upright position until inoculums is absorbed by medium, then plates was inverted and incubated aerobically for 48 hour at 37° C. typical colonies have white-cream color, 1-1.5 mm in diameter (Camschella, et al., 1998), (Rybka and Kailasapathy, 1996). Calculate the average of colonies in duplicate plates of appropriate dilution and multiply by dilution factor and ten (cell count = average

of colonies x dilution factor x 10). To get the cell count of *S. thermophilus* / gm of test sample. *L. bulgaricus* was enumerated by aseptically transferring 0.1 ml of appropriate dilution to each of duplicate MRS agar, supplemented with acetic acid until the pH of the medium reach 5.4 (acidified MRS), inoculums was spread over surface of agar using sterile bent glass streaking rods, avoiding extreme edges of the plate, plates then retained in upright position until inoculums is absorbed by medium then Inverted and incubated in anaerobic jars for 72 hour at 37º C. typical colonies have lenticular often sharp-shaped forms with 1-3 mm in diameter (National Yogurt Association (2008), International Dairy Federation Standard 117: 1988). Calculate the average of colonies in duplicate plates of appropriate dilution and multiply by dilution factor and ten (cell count = average of colonies x dilution factor x 10). To get the cell count of *L. bulgaricus* / gm of test sample.

3.2.4.: Growth interaction experiments:

All experiments were done in duplicate, Gram stain was used to confirm the identity of each bacteria grown in the enumeration medium.

a. Growth interaction between *B. lactis* and yoghurt LAB:

Approximately 6 log_{10} of each of *B. lactis, S. thermophilus* and *L. bulgaricus* / ml was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6

days, 9 days and 12 days from the time of inoculation. Each bacterium was enumerated as in the growth experiments.

b. Growth inhibition interaction between *S. aureus* and yoghurt LAB:

Approximately 6 $\log_{10}$ of each of *S. aureus, S. thermophilus* and *L. bulgaricus* / ml was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days, and 12 days from the time of inoculation. Then the experiment was repeated by inoculating approximately 7 $\log_{10}$ of each of *S. aureus*, *S. thermophilus* and *L. bulgaricus* / ml (log 7 *S. aureus*, obtained by inoculating 1.2 ml of BHI broth which has been inoculated with one colony of enterotoxigenic *S. aureus* isolate and incubated for 24 hour at 35-37° C; log 7 yoghurt LAB, obtained by adding two botch of mixture culture of LAB to 400 liter pasteurized milk) to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days and 6

days from the time of inoculation. Each bacterium was enumerated as in the growth experiments.

c. Growth inhibition interaction between *S. aureus* and *B. lactis*:

Approximately 6 $\log_{10}$ of each of *S. aureus* and *B. lactis* / ml was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days and 12 days from the time of inoculation. Then the experiment was repeated by inoculating approximately 7 $\log_{10}$ of each *S. aureus* and *B. lactis* / ml (log 7 *B. lactis* obtained by taking one ml of 10^{-10} serial dilution of *B. lactis* culture powder) to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days and 6 days from the time of inoculation. Each bacterium was enumerated as in the growth experiments.

d. Growth inhibition interaction between *S. aureus, B. lactis* and yoghurt LAB:

Approximately 6 log_{10} of each of *S. aureus, B. lactis S. thermophilus* and *L. bulgaricus* / ml was inoculated and mixed thoroughly to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days, 6 days, 9 days and 12 days from the time of inoculation. Then the experiment was repeated by inoculating approximately 7 log_{10} of each bacterium / ml to each of two containers of one liter fresh pasteurized cow's milk. After that the two containers were incubated at 45° C for 4 hour (the count of each bacterium was monitored initially then after 4 hour; end of incubation fermentation period), one container then was stored at room temperature (20-25° C, Sample 1) and the second was stored at refrigeration temperature (4° C, Sample 2). Count of each bacterium was monitored then initially (same count at the end of incubation period), 8 hour (4 hour from starting storage period), 24 hour (20 hour from starting storage period) , 3 days and 6 days from the time of inoculation. Each bacterium was enumerated as in the growth experiments.

3.2.5.: Preparation of serial dilution of test samples:

Ten grams of each sample was transferred aseptically into dilution bottles containing 90 ml sterilized peptone water and mixed thoroughly (10^{-1} dilution), then serial dilutions were made by transfer one ml of each dilution into test tubes containing 9 ml sterilized peptone

water. Appropriate dilution of each sample was spread over Petri dishes containing the required media for enumeration of each bacterium. (Rybka and Kailasapathy, 1996).

3.2.6.: Statistical analysis:

Fisher protected t-test at $p<0.01$ was performed for significant effects of means of different factors.

4. Results and Discussion:

4.1.: Isolation and characterization of *S. aureus*:

S. aureus was isolated from 16 different food samples including (white cheese, cow's milk, chicken, lamb and beef meat) purchased from local market. Colonies of *S. aureus* over BPA after 48hr incubation at 37° C were found to be typically circular, smooth, convex, moist, 2-3 mm in diameter, gray-black to jet black, frequently with light colored (off-white) margins, surrounded by opaque zone (precipitate), and frequently with outer clear zone. The identity of *S. aureus* was confirmed by being Gram positive and *cocci* (arrangement as mono-*cocci*, di-*cocci*, tri- *cocci* and clusters). The ability of *S. aureus* to produce enterotoxins includes tests for catalase, coagulase and DNase enzymes (Table 1). It was found that isolates from all food samples were catalase positive. In addition that all isolates from 4 out of 5 white cheese samples were coagulase and DNase positive. Jay. (2000) reported that 93% of enterotoxigenic strains of *S. aureus* produce coagulase, and 95% produce DNase. Cliver and Riemann (2002) reported that coagulase positive *S. aureus* are able to produce enterotoxins.

An isolate of *S. aureus* from white cheese (number 14) was found to be the most active clot forming isolate; it's given the symbol (14) which is used in the experimentation thereafter.

Table 1: Tests to confirm the ability of *S. aureus* isolated from different food samples to produce enterotoxins*.

Isolate number	Food sample	Catalase test	Coagulase test	DNase test
1	Cow's milk	+	-	-
2	Cow's milk	+	-	-
3	Cow's milk	+	-	-
4	Cow's milk	+	-	-
5	Cow's milk	+	-	-
6	Lamb meat	+	-	-
7	Lamb meat	+	-	-
8	beef meat	+	-	-
9	beef meat	+	-	-
10	Chicken meat	+	-	-
11	Chicken meat	+	-	-
12	White cheese	+	++	+
13	White cheese	+	++	+
14	White cheese	+	++++	+
15	White cheese	+	-	-
16	White cheese	+	+++	+

(*), one isolate from each food sample; (+), positive; (-), negative.

4.2.: Determination of *S. aureus* cell count:

For the determination of *S. aureus* count to be used in the interaction and antagonism experimentation with LAB starter culture and with *B. lactis*, quadruplicate test tubes containing sterilized BHI broth with pure colony of *S. aureus* (14) each were incubated at 37° C for 24 hour. Serial dilutions of the culture up to 10^{-9} were made; one ml of each dilution was spread over triplicate solidified BPA and incubated at 37° C for 48 hour. The results indicated that the log numbers were 9.48 $\log_{10}$, 9.3 $\log_{10}$, 9.7 $\log_{10}$, 9.85 $\log_{10}$ with an average of 9.6 $\log_{10}$ / ml of BHI broth. This procedure of determining the log number of *S. aureus* was used in all progressive experimentation.

4.3.: Growth experiments of bacteria in milk:

Results are averages of duplicate samples.

4.3.1.: *S. aureus* growth in milk:

In an experiment of the survival of the enterotoxigenic *S. aureus* (14) in fresh pasteurized cow's milk expressed in $\log_{10}$ / ml on semi log paper during incubation temperature of 45° C for 4 hour and thereafter storage period in room and refrigeration temperatures for a period of 12 days with changes of pH values is presented in Table (2) and Figure (1). Inoculum of approximately 9 $\log_{10}$ of *S. aureus* (14) was transferred into each of two plastic containers of one liter fresh pasteurized cow's milk. The initial count was 6.78 $\log_{10}$ / ml with a pH value of 6.64 before incubation period of 4 hour at 45° C (the period required for milk fermentation to yoghurt). No significant increase in the *S. aureus* count (6.89 $\log_{10}$ / ml) with slight decrease in the pH value (6.24). This result indicates that no multiplications of the bacterial cell during the incubation period which may be due to the lag phase required for this bacterium before cell division. This is in addition to the higher temperature of incubation (45°C) which within the maximum temperature of growth of this bacteria. After transferring milk sample (1) to room temperature and sample (2) to refrigeration temperature the log number of *S. aureus* after 4 hour of storage (8 hour from the begging of inoculation) slightly increased (7.11 $\log_{10}$), and to 7.62 $\log_{10}$ after 20 hour of storage. The count of *S. aureus* started decreasing after 3 days (6.82 $\log_{10}$), 6 days (5.7 $\log_{10}$), 9 days (4.49 $\log_{10}$) and the lowest was after 12 days (2.86 $\log_{10}$), pH values decreased from 6.24 after 4 hour of incubation at 45° C to 4.94 after 8 hour from the begging of inoculation reaching the minimum of 3.93 after 12 days of storage at room temperature. This decrease in pH value is probably due to the production of lactic acid or other organic acid as a result of the growth of pasteurization resistant bacteria, or due to

production of organic acid by *S. aureus* itself as was reported by (Fujikawa and Morozumi, 2006). This may also reflect the decrease in the count of *S. aureus* in milk during this period. No significant change in the count of *S. aureus* in milk during refrigeration storage which ranging between 7.5- 7.9 $\log_{10}$. This increase may be due to the transitional period after fermentation period and before reaching refrigeration temperature. *S. aureus* survived and still viable during refrigeration temperature since refrigeration temperature seems to suppress the growth and multiplication of *S. aureus* as well as other mesophilic and thermophilic bacteria. Similarly no significant changes in the pH value in milk sample number (2) during refrigeration storage period. It was found that the effect of pH value of the medium on the survival of *S. aureus* during storage in this experiment was highly significant ($P<0.01$).

Table 2: Survival (log_{10} / ml) of enterotoxigenic *S. aureus* isolate (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Incubation period at 45° C	Sample 1		Sample 2	
	log_{10} / ml *S. aureus*	pH	log_{10} / ml *S. aureus*	pH
0 hr	6.78	6.64	6.78	6.64
4 hr	6.89	6.24	6.89	6.24
Storage period	Room temperature (20-25° C)		Refrigeration temperature (4° C)	
0 hr	6.89	6.24	6.89	6.24
4 hr	7.11	4.94	7.45	5.95
20 hr	7.62	4.45	7.9	5.83
3 days	6.82	4.23	7.62	5.82
6 days	5.7	4.11	7.85	5.6
9 days	4.49	3.97	7.74	5.47
12 days	2.86	3.93	7.79	5.41

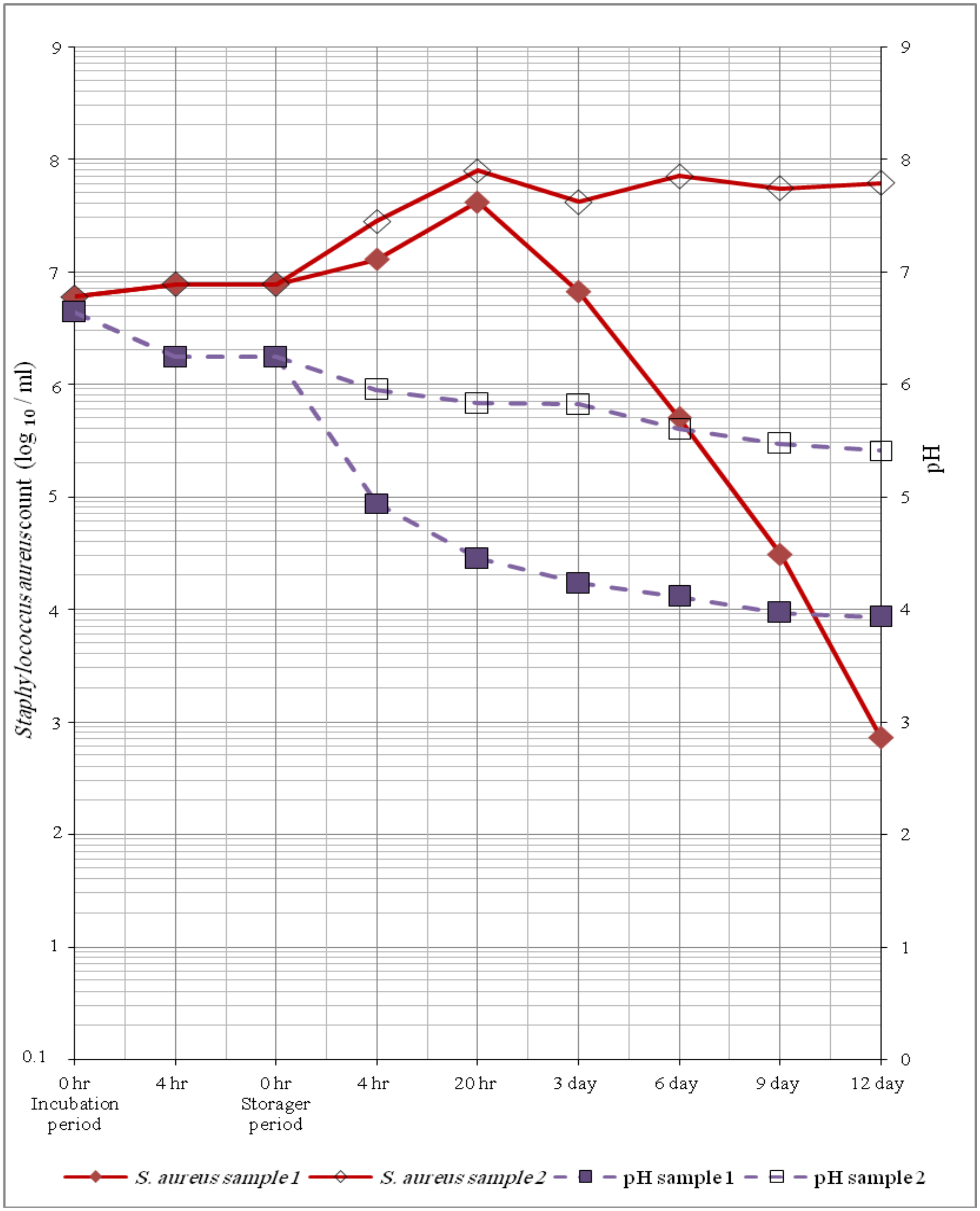


Figure 1: Survival (log / ml) of enterotoxigenic *S. aureus* isolate (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.3.2.: *B. lactis* growth in milk:

The growth dynamic of *B. lactis* during incubation period of 4 hour at 45° C (fermentation period), then storage at room and refrigeration temperatures for a period of 12 days with the changes of pH values is presented in Table (3) and Figure (2). Initial inoculum of approximately 9 $\log_{10}$ was inoculated to each of two plastic containers containing one liter fresh pasteurized cow's milk. The initial count of *B. lactis* was 6.83 $\log_{10}$ / ml and the initial pH value was 6.55, *B. lactis* count slightly increased during the fermentation period reaching 7.56 $\log_{10}$/ ml and the pH value was slightly decreased to about 6.09. This result indicates that *B. lactis* was activated and start multiplication during the fermentation period which results in the production of lactic acid causing the slight decrease in the pH value. *B. lactis* count was significantly increased ($P< 0.01$) during storage period at room temperature to 7.91 $\log_{10}$/ ml after 4 hour of storage reaching maximum of 8.98 $\log_{10}$/ ml (>2 $\log_{10}$/ ml) after 12 days. pH values continued decreasing to 5.69 after 4 hour of storage reaching 4.02 after 12 days of storage. On the other hand, the count of *B. lactis* in sample (2) which was stored in refrigeration temperature was not significantly changed during storage period ranging from 7.6 $\log_{10}$ and 7.87 $\log_{10}$, with no significant change in pH value (ranging from 6.05 and 5.99). Refrigeration temperature suppressed the growth of *B. lactis* without decreasing the bacterial count.

Table 3: Growth dynamic (log_{10} / ml) of *B. lactis* in fresh pasteurized cow's milk after 4 hour incubation at 45° C. stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Fermentation period at 45° C	Sample 1		Sample 2	
	log_{10} / ml *B. lactis*	pH	log_{10} / ml *B. lactis*	pH
0 hr	6.83	6.55	6.83	6.55
4 hr	7.56	6.09	7.56	6.09
Storage period	Room temperature (20-25° C)		Refrigeration temperature (4° C)	
0 hr	7.56	6.09	7.56	6.09
4 hr	7.81	5.69	7.63	6.05
20 hr	7.93	5.03	7.65	6.02
3 days	8.6	4.42	7.87	6.04
6 days	8.86	4.12	7.62	6.07
9 days	8.98	4.07	7.71	6.02
12 days	8.78	4.02	7.6	5.99

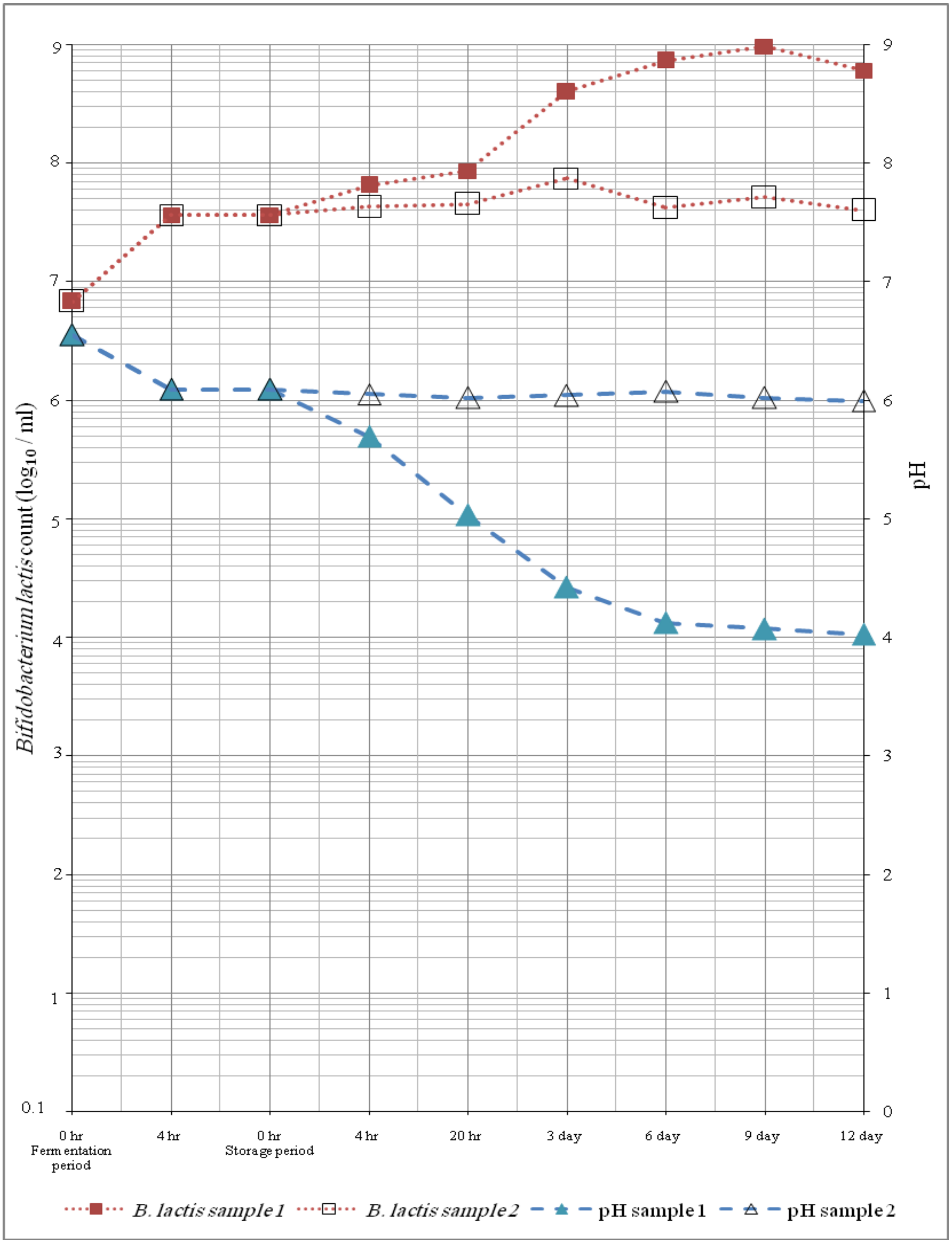


Figure 2: Growth dynamic (log / ml) of *B. lactis* in fresh pasteurized cow's milk after 4 hour incubation at 45° C. stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.3.3.: Yoghurt lactic acid bacteria growth in milk:

Yoghurt LAB starter culture (*S. thermophilus* and *L. bulgaricus*) growth dynamics in fresh pasteurized cow's milk during fermentation period for yoghurt manufacturing and thereafter storage period of 12 days in room and refrigeration temperatures with the pH value changes during the same period is presented in Table (4) and Figure (3). The count of each of the two LAB species (*S. thermophilus* and *L. bulgaricus*) increased from 6.86 and 6.92 log_{10} initially to 7.49 and 7.62 log_{10} during the fermentation period, respectively. The pH values significantly decrease ($P< 0.01$) from 6.64 initially to about 4.55 as a result of lactic acid production. During storage at room temperature *S. thermophilus* and *L. bulgaricus* count continued increasing to 7.72 and 7.88 log_{10} after 4 hour of storage, 7.77 and 7.92 log_{10} after 20 hour of storage, and reaching maximum counts of 8.1 and 8.3 log_{10} after 3 days of storage, respectively. The pH values continue decreasing to reach 4.19 after 3 days of storage. In refrigeration temperature (Sample 2) *S. thermophilus* and *L. bulgaricus* counts did not significantly changed, ranging from 7.28 to 7.61 log_{10} and 7.47 to 7.8 log_{10}, respectively. While the pH values continue decreasing to reach about 4.17 at the end of storage period, this result shows that refrigeration temperature suppressed the growth of LAB.

Table 4: Growth dynamics (log_{10} / ml) of LAB (*S. thermophilus* and *L. bulgaricus*) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Fermentation period at 45° C	Sample 1			Sample 2		
	log_{10} / ml *S. thermophilus*	log_{10} / ml *L. bulgaricus*	pH	log_{10} / ml *S. thermophilus*	log_{10} / ml *L. bulgaricus*	pH
0 hr	6.86	6.92	6.64	6.86	6.92	6.64
4 hr	7.49	7.62	4.55	7.49	7.62	4.55
Storage period	Room temperature (20-25° C)			Refrigeration temperature (4° C)		
0 hr	7.49	7.62	4.55	7.49	7.62	4.55
4 hr	7.72	7.88	4.41	7.63	7.79	4.43
20 hr	7.77	7.92	4.23	7.61	7.8	4.37
3 days	8.1	8.3	4.19	7.59	7.79	4.35
6 days	-*	-*	-*	7.54	7.76	4.33
9 days	-*	-*	-*	7.28	7.57	4.27
12 days	-*	-*	-*	7.38	7.47	4.17

(*) Samples contaminated with fungi.

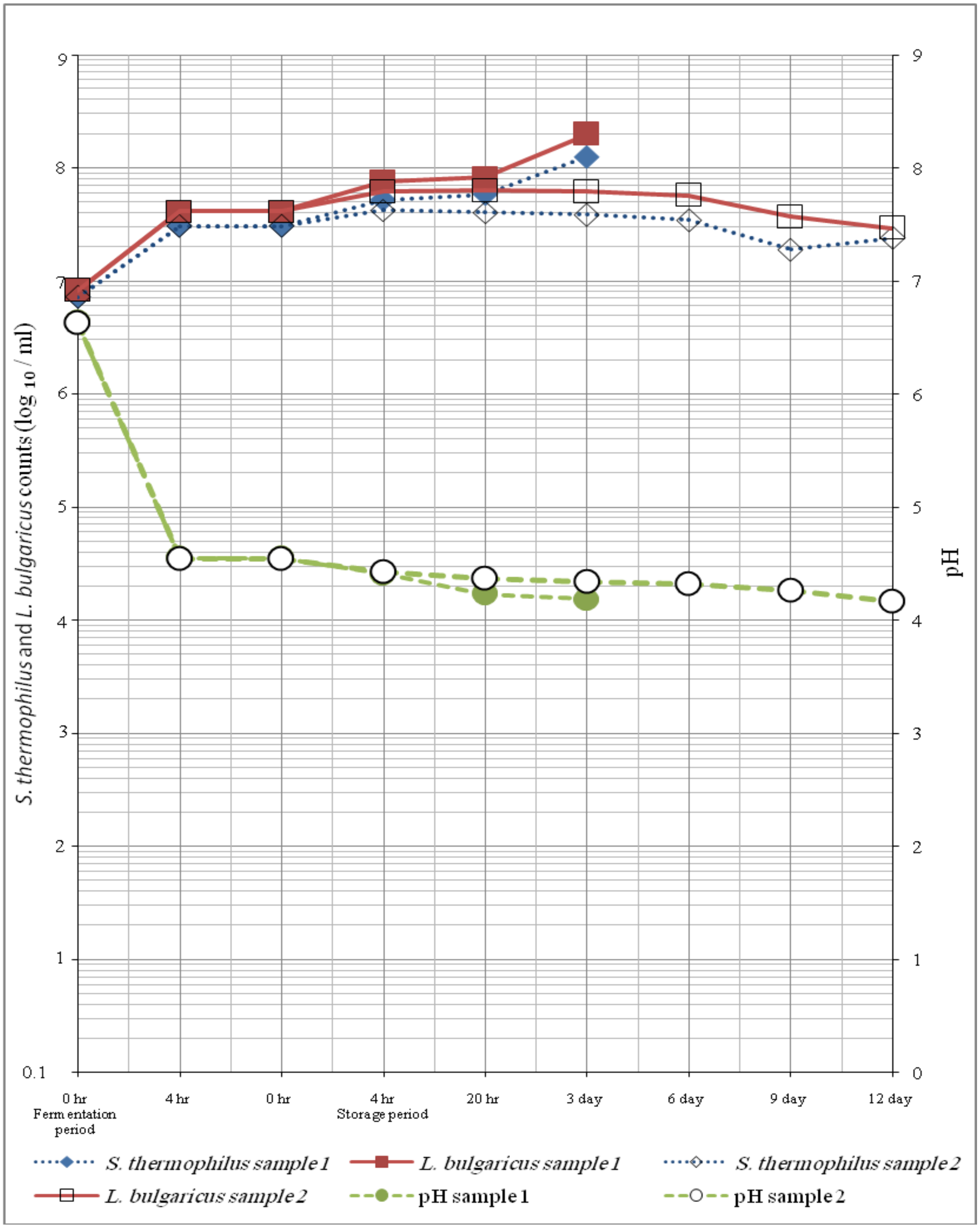


Figure 3: Growth dynamics (log_{10} / ml) of LAB (*S. thermophilus* and *L. bulgaricus*) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.4.: Growth interaction experiments:

4.4.1.: Growth interaction between *B. lactis* and yoghurt LAB starter culture in milk:

The growth interaction between the LAB starter cultures (*S. thermophilus* and *L. bulgaricus*) and the probiotic bacteria *B. lactis* during the fermentation period and storage period thereafter at room and refrigeration temperatures for 12 days and the pH value changes is presented in Table (5) and Figure (4). The results show an increase of approximately of 0.5 $\log_{10}$ of the three bacteria from the initial count during the 4 hour fermentation period with significant decrease ($P < 0.01$) in the pH value (initially 6.55 to about 4.79). Except the slight increase in the number of *S. thermophilus* and *L. bulgaricus* (up to 1.5 $\log_{10}$ / ml after 3 days of storage at room temperature) no significant further increase after fermentation in the growth of *S. thermophilus* and *L. bulgaricus* and *B. lactis* of the two fermented milk samples stored at both room and refrigeration temperatures. This result shows that there is no antagonistic effect between the three bacteria in fermented milk during fermentation and storage period of 12 days. The beneficial human health value of the probiotic *B. lactis* therefore is not affected. Also Tabasco et al. (2007) repoted that *B. lactis* and LAB (*S. thermophilus* and *L. bulgaricus*) counts in fermented milk remain constant during storage period of 4 weeks at refrigeration temperature of 4° C.

Table 5: Growth interaction (log_{10} / ml) between yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) and *B. lactis* in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Fermentation period at 45° C	Sample 1				Sample 2			
	log_{10} / ml *B. lactis*	log_{10} / ml *S. thermophilus*	log_{10} / ml *L. bulgaricus*	pH	log_{10} / ml *B. lactis*	log_{10} / ml *S. thermophilus*	log_{10} / ml *L. bulgaricus*	pH
0 hr	6.83	6.8	6.91	6.55	6.83	6.8	6.91	6.55
4 hr	7.4	7.34	7.46	4.79	7.4	7.34	7.46	4.79
Storage period	Room temperature (20-25° C)				Refrigeration temperature (4° C)			
0 hr	7.4	7.34	7.46	4.79	7.4	7.34	7.46	4.79
4 hr	7.65	7.72	7.74	4.4	7.57	7.63	7.72	4.42
20 hr	7.76	7.81	7.86	4.19	7.69	7.61	7.72	4.28
3 day	7.24	8.86	8.55	4.1	7.65	7.59	7.72	4.26
6 day	7.53	8.34	8.73	4.08	7.18	7.56	7.66	4.24
9 day	7.71	8.43	8.69	4.06	7.51	7.58	7.62	4.22
12 day	7.43	8.46	8.85	4.08	7.48	7.36	7.71	4.17

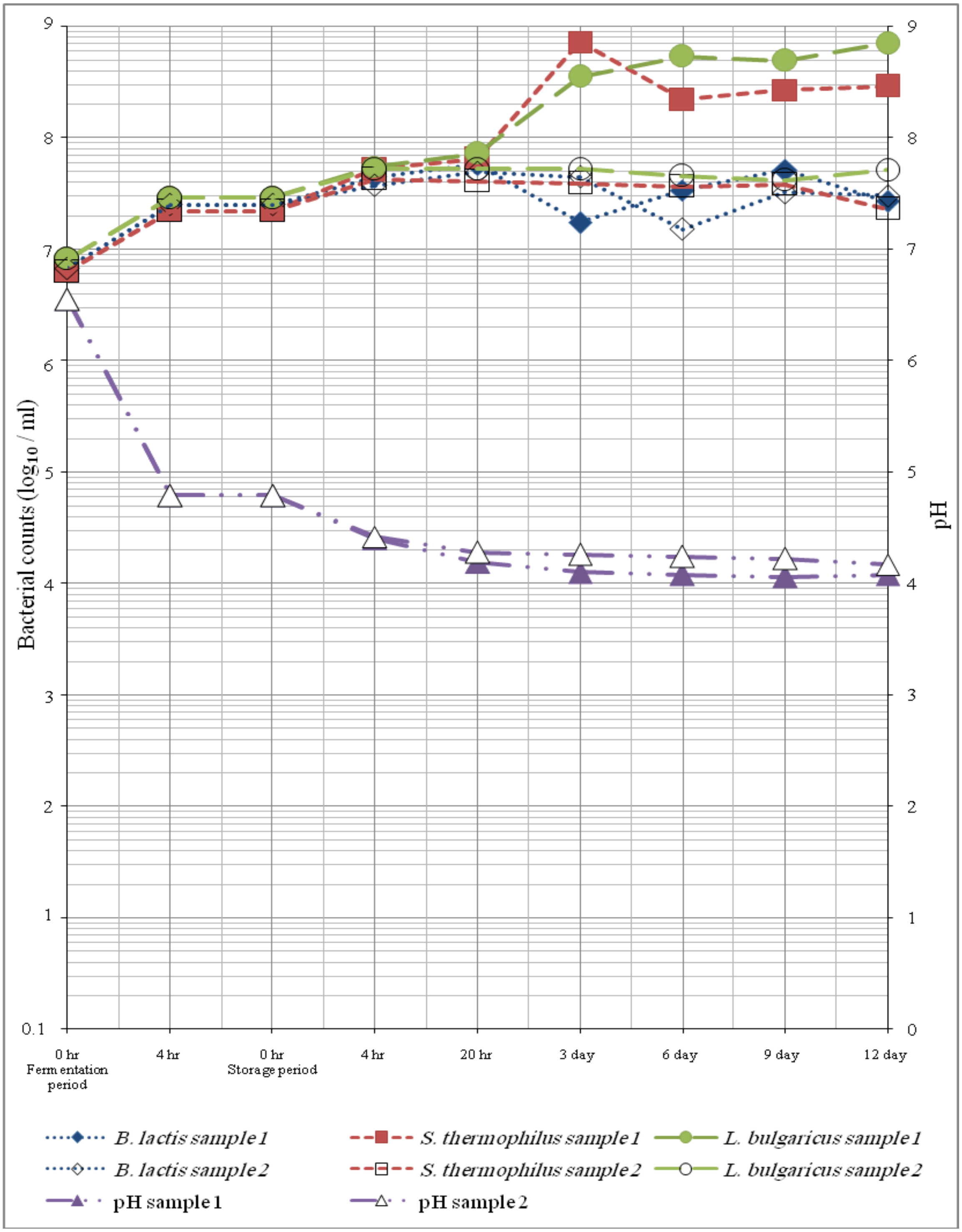


Figure 4: Growth interaction (log_{10} / ml) between yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) and *B. lactis* in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.4.2.: Growth inhibition interaction between *S. aureus* and yoghurt LAB starter culture in milk:

The growth inhibition interaction between yoghurt LAB starter culture (*S. thermophilus* and *L. bulgaricus*) and *S. aureus* (14) and the pH value changes in fresh pasteurized cow's milk during fermentation process and extended storage of 12 days in room and refrigeration temperatures is presented in Table (6) and Figure (5). It was found that the viable cell count of *S. aureus* (14) slightly decreased from an initial number of 6.62 log_{10} to 6.36 log_{10} during fermentation period, and significantly ($P<0.01$) decrease during storage period with complete inhibition after 9 days of storage at room temperature and 12 days of storage at refrigeration. pH values were also significantly ($P<0.01$) decreased during fermentation period and slightly decreased during storage period at both room and refrigeration temperatures. On the other hand, LAB starter culture counts were slightly increased (0.5 log_{10}) during fermentation period and significantly ($P<0.01$) increased 1.5 log_{10} during storage period at room temperature with no significant changes in their count during refrigeration storage. From these results the antagonistic effect of yoghurt LAB starter culture (*S. thermophilus* and *L. bulgaricus*) on *S. aureus* (14) probably was due to the decrease in the pH value from initially 6.64 to 3.93 at day 9 of storage at room temperature and 4.02 after 12 days of storage at refrigeration. This decrease in pH value as a result of breakdown of lactose in milk into lactic acid, the competition on nutrient and possible bacteriocin production by yoghurt LAB have been contributing to the control and completely inhibition of the growth of *S. aureus* (14) fermented milk.

Charlier et al. (2009) and Le Marc et al. (2009) suggested that the inhibition of *S. aureus* by LAB starter culture is related to many antagonistic factors including decrease in pH as a consequence of lactic acid production, competition for nutrient, production of H_2O_2 and

production and accumulation of anti *Staphylococcal* substances such as bacteriocin. Similar additional experiment was designed by increasing the initial inoculums by approximately one $\log_{10}$ of yoghurt LAB starter culture and *S. aureus* (14), resulted in generally similar trend of antagonistic effect between yoghurt LAB starter culture and *S. aureus* (14) during fermentation period and extended storage for 6 days at both room and refrigeration temperatures (Appendix C, Table 1) .

Table 6: Growth interaction ($\log_{10}$ / ml) between yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) and *S. aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Fermentation period at 45° C	Sample 1				Sample 2			
	Log $_{10}$ / ml *S. therm-ophilus*	Log $_{10}$ / ml *L. bulg-aricus*	Log $_{10}$ / ml *S. aureus*	pH	Log $_{10}$ / ml *S. therm-ophilus*	Log $_{10}$ / ml *L. bulg-aricus*	Log $_{10}$ / ml *S. aureus*	pH
0 hr	6.86	6.92	6.62^{a}	6.64	6.86	6.92	6.62^{a}	6.64
4 hr	7.34	7.53	6.36^{a}	4.55	7.34	7.53	6.36^{a}	4.55
Storage period	Room temperature (20-25° C)				Refrigeration temperature (4° C)			
0 hr	7.34	7.53	6.36^{a}	4.55	7.34	7.53	6.36^{a}	4.55
4 hr	7.52	7.81	5.9^{b}	4.34	7.44	7.76	5.94^{b}	4.39
20 hr	7.61	7.83	5.04^{c}	4.31	7.4	7.76	4.9^{c}	4.35
3 day	8.25	8.13	2.57^{d}	4.19	7.36	7.72	3.07^{d}	4.33
6 day	8.49	8.4	1.2^{e}	4.05	7.32	7.73	2.34^{e}	4.29
9 day	7.83	7.94	0^{f}	3.93	7.08	7.58	1.61^{f}	4.04
12 day	7.11	7.59	0^{f}	3.88	7.28	7.51	0^{g}	4.02

a-g: means within columns with different superscripts are different at $P < 0.01$

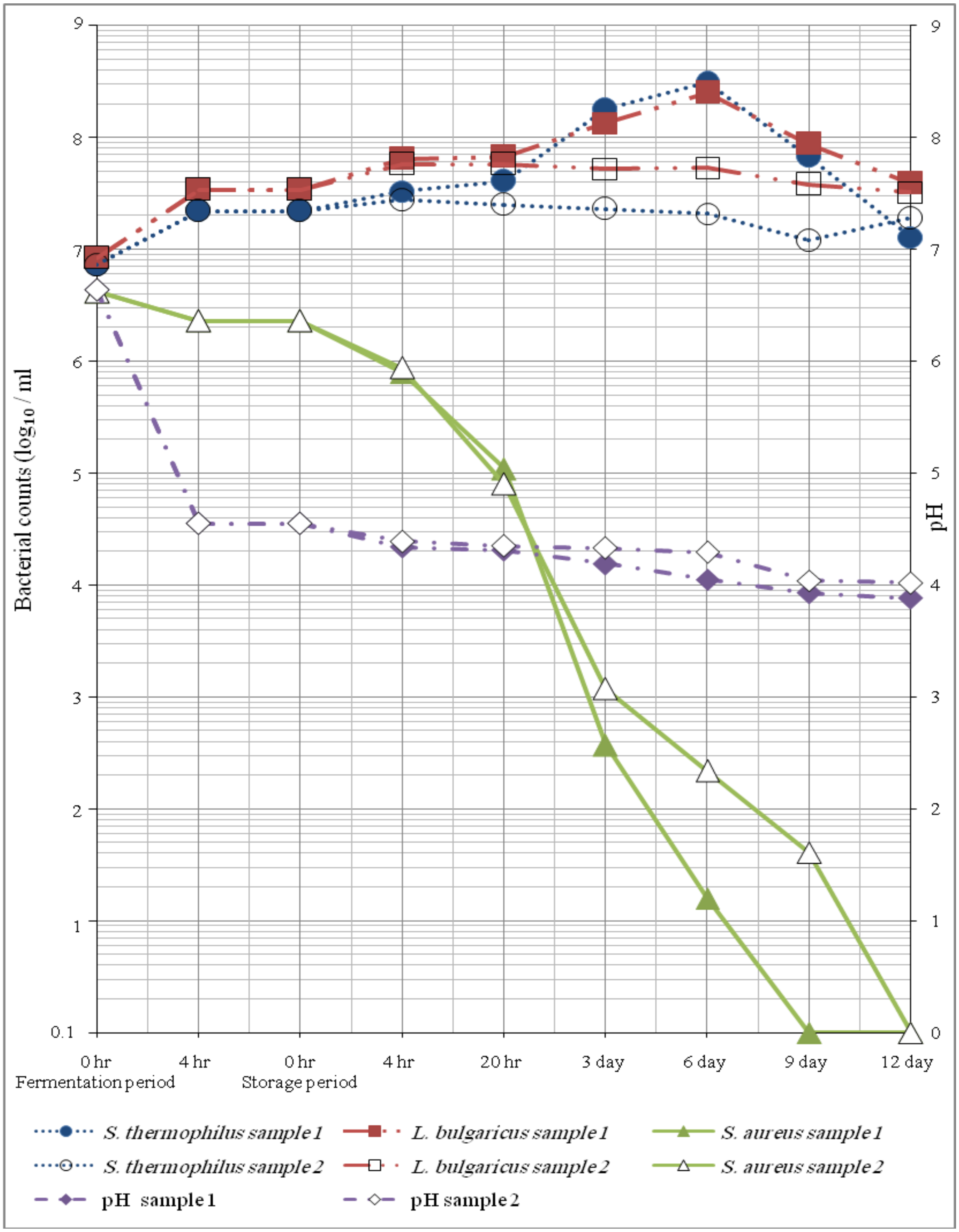


Figure 5: Growth interaction (log_{10} / ml) between yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) and *Staphylococcus aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.4.3.: Growth inhibition interaction between *S. aureus* and *B. lactis* in milk:

The results of the experiment of growth interaction between *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk during fermentation period and thereafter storage for a period of 12 days at room and refrigeration temperatures are presented in Table (7) and Figure (6). These results show a slight decrease in the count of *S. aureu*s (14) (0.37 $\log_{10}$) and increase in the growth of *B. lactis* (0.63 $\log_{10}$) with a slight decrease in the pH value from 6.55 initially to 6.09 after 4 hour fermentation period, during storage at room temperature thereafter *S. aureus* (14) count was significantly ($P<0.01$) decrease to reach 2.9 $\log_{10}$ after 3 days with complete inhibition after 6 days of storage. The growth of *B. lactis* continue to increase in count reaching maximum of 8.94 $\log_{10}$ after 9 days of storage, pH value was significantly ($P<0.01$) decreased to its minimum of 4.11. At refrigeration temperature *S. aureus* (14) count decreased significantly ($P<0.01$) to a minimum of 2.6 $\log_{10}$ after 6 days of storage and complete inhibition after 9 days of storage, *B. lactis* count continued to be approximately the same throughout the storage period with no significant decrease in the pH value. The result of this experiment shows an antagonistic effect by *B. lactis* against *S. aureus* (14) in both samples stored at room and refrigeration temperatures.

Similar to the effect of LAB starter culture against *S. aureus* (14), *B. lactis* effect may be due to competition of nutrient resulted in starvation of *S. aureus* (14), lactic acid production and possible bacteriocin production by *B. lactis*. The effect of this antagonism on *S. aureus* (14) in refrigeration is relatively less, which is probably due to the slower metabolic activity of *B. lactis*. This experiment shows that in addition to the beneficial human health value of *B. lactis* added to fermented milk products as a probiotic, it's also considered as anti *S. aureus* (14) and may be other pathogens growth in the fermented milk and products.

In a separate similar experiment conducted by increasing the initial count of both *B. lactis* and *S. aureus* (14) by one log to find out the effect of the initial number on the antagonistic effect of *B. lactis* on *S. aureus* (14). It was found that the same antagonistic effect of the previous experiment during fermentation and extended storage period of 6 days in both room and refrigeration temperatures (Appendix C, Table 2).

The conclusion of this experiment is that the probiotic bacteria *B. lactis* strongly inhibited the growth of the enterotoxigenic *S. aureus* (14) in fermented milk during storage. Lahtinen et al. (2007) reported that the inhibitory effect of *B. lactis* against *S. aureus* is related to hydrogen peroxide formation and possible production of heat stable proteinaceous compounds were the pH controlled supernatants of *B. lactis* did not show antimicrobial activity against *S. aureus*. It's may be of value to experiment the main factor that inhibit the *S. aureus* growth by the probiotic bacteria *B. lactis* in milk and to experiment the effect of the probiotic bacteria *B. lactis* on the growth of other pathogenic bacteria in milk and milk products or other processed food in the future.

Table 7: Growth interaction (log_{10} / ml) between *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

Fermentation period at 45° C	Sample 1			Sample 2		
	Log $_{10}$ / ml *B. lactis*	Log $_{10}$ / ml *S. aureus*	pH	Log $_{10}$ / ml *B. lactis*	Log $_{10}$ / ml *S. aureus*	pH
0 hr	6.83	6.62^{a}	6.55	6.83	6.62^{a}	6.55
4 hr	7.46	6.25^{b}	6.09	7.46	6.25^{b}	6.09
Storage period	Room temperature (20-25° C)			Refrigeration temperature (4° C)		
0 hr	7.46	6.25^{b}	6.09	7.46	6.25^{b}	6.09
4 hr	7.62	5.98^{c}	5.57	7.56	5.88^{c}	5.76
20 hr	7.89	4.78^{d}	4.33	7.57	5.49^{d}	5.68
3 day	8.35	2.9^{e}	4.25	7.53	4.15^{e}	5.63
6 day	8.78	0^{f}	4.13	7.4	2.63^{f}	5.6
9 day	8.94	0^{f}	4.11	7.62	0^{g}	5.58
12 day	-	-	-	7.6	0^{g}	5.55

a-g: means within columns with different superscripts are different at $P < 0.01$

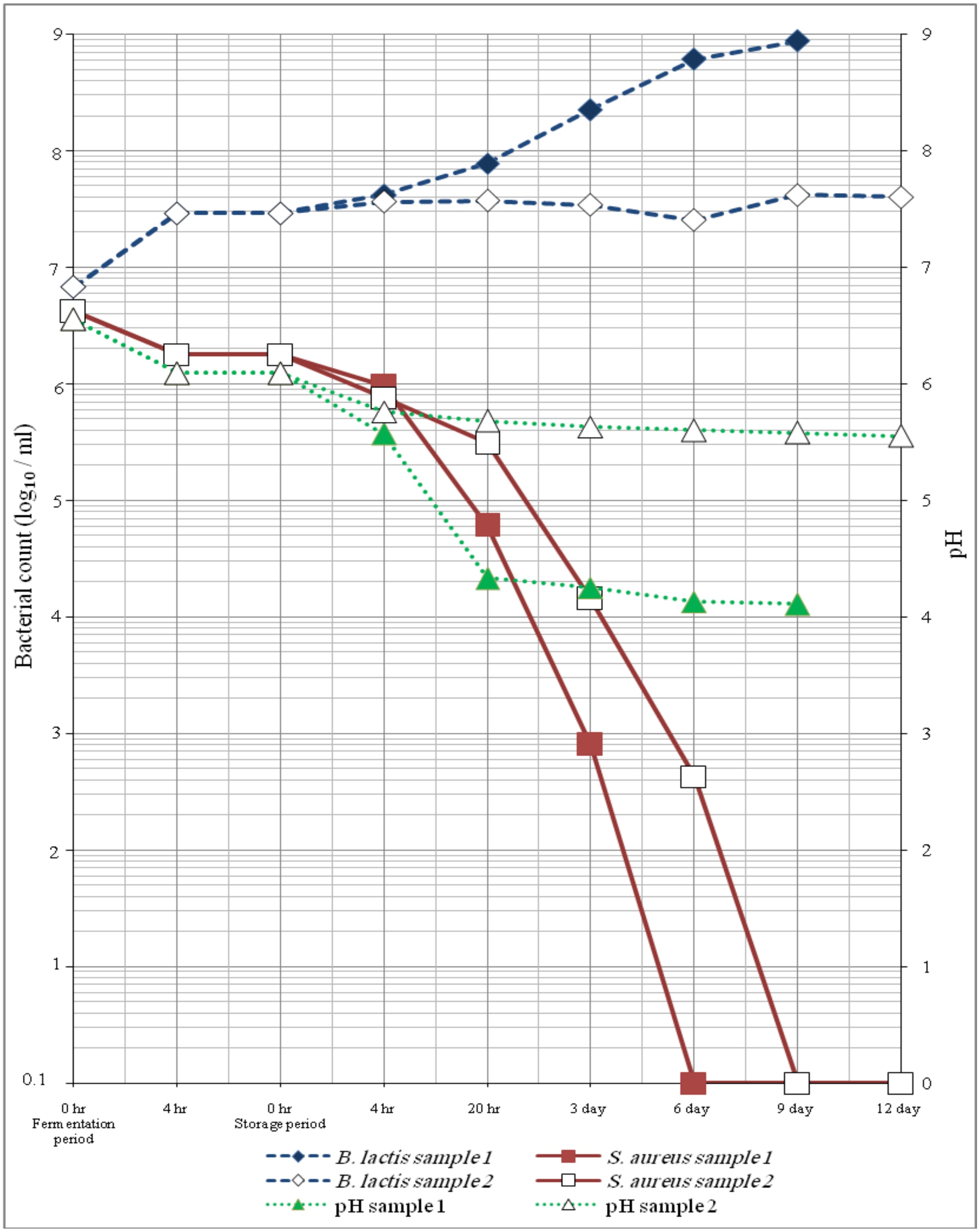


Figure 6: Growth interaction (log_{10} / ml) between *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 12 days.

4.4.4.: Growth inhibition interaction between *S. aureus, B. lactis* and yoghurt LAB starter culture in milk:

The combined growth interaction effect of yoghurt LAB starter culture (*S. thermophilus* and *L. bulgaricus*) and *B. lactis* against *S. aureus* (14) growth during milk fermentation to yoghurt and storage period of 9 days at room and refrigeration temperatures is presented in Table (8) and Figure (7). In general similar antagonistic effect of the combined yoghurt LAB starter culture and *B. lactis* against *S. aureus* (14) in fermented milk during fermentation period and throughout storage period for both samples stored at room and refrigeration temperatures for 9 days as they are separately. The combined antagonistic effect of these bacteria on the growth of *S. aureus* (14) shows that the *S. aureus* (14) count significantly ($P<0.01$) decreased reaching a complete inhibition after 6 days and 9 days of storage at room and refrigeration temperatures, respectively. In the previous experiments of the effect of LAB against *S. aureus* (14) pointed out that complete inhibition of *S. aureus* (14) was after 9 days of storage at room temperature and 12 days of storage at refrigeration temperature. Whereas complete inhibition of *S. aureus* (14) by *B. lactis* was reached after 6 days of storage at room temperature and 9 days of storage at refrigeration temperature. Lower count of *S. aureus* (14) 1.76 $\log_{10}$ and 2.8 $\log_{10}$ with samples stored for 3 days at room and refrigeration temperatures, compared with the growth number of the antagonistic effect of yoghurt LAB (2.57 $\log_{10}$ and 3.07 $\log_{10}$) and (2.9 $\log_{10}$ and 4.15 $\log_{10}$) with *B. lactis*, respectively.

Increasing the initial number of all bacteria by approximately one log did not significantly ($P<0.01$) affect the trend of antagonistic effect against *S. aureus* (14) compared to previous experiments (Appendix C, Table 3). As a result of all growth inhibition interaction experimentations between yoghurt LAB starter culture or *B. lactis*

each alone or combined together both yoghurt LAB starter culture and *B. lactis* against *S. aureus* (14) all were able to control and consequently completely inhibited the growth of *S. aureus* (14) during fermentation process and throughout the extended storage period at room and refrigeration temperatures.

Table 8: Growth interaction (log_{10} / ml) between yoghurt LAB starter culture, *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 9 days.

Fermentation period at 45° C	Sample 1					Sample 2				
	Log_{10} / ml *B. lactis*	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH	Log_{10} / ml *B. lactis*	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH
0 hr	6.83	6.8	6.91	6.62^{a}	6.55	6.83	6.8	6.91	6.62^{a}	6.55
4 hr	7.36	7.28	7.32	5.96^{a}	4.82	7.36	7.28	7.32	5.96^{a}	4.82
Storage period	Room temperature (20-25° C)					Refrigeration temperature (4° C)				
0 hr	7.36	7.28	7.32	5.96^{a}	4.82	7.36	7.28	7.32	5.96^{a}	4.82
4 hr	7.57	7.61	7.68	5.38^{b}	4.33	7.49	7.56	7.63	5.52^{b}	4.39
20 hr	7.69	7.72	7.81	4.04^{c}	4.16	7.58	7.57	7.61	4.45^{c}	4.29
3 day	7.9	7.67	7.24	1.76^{d}	4.15	7.51	7.52	7.62	2.8^{d}	4.26
6 day	7.42	8.28	8.11	0^{e}	4.11	7.23	7.28	7.43	1.05^{e}	4.23
9 day	7.72	8.19	8.33	0^{e}	4.09	7.42	7.32	7.38	0^{f}	4.24

a-f: means within columns with different superscripts are different at $P < 0.01$

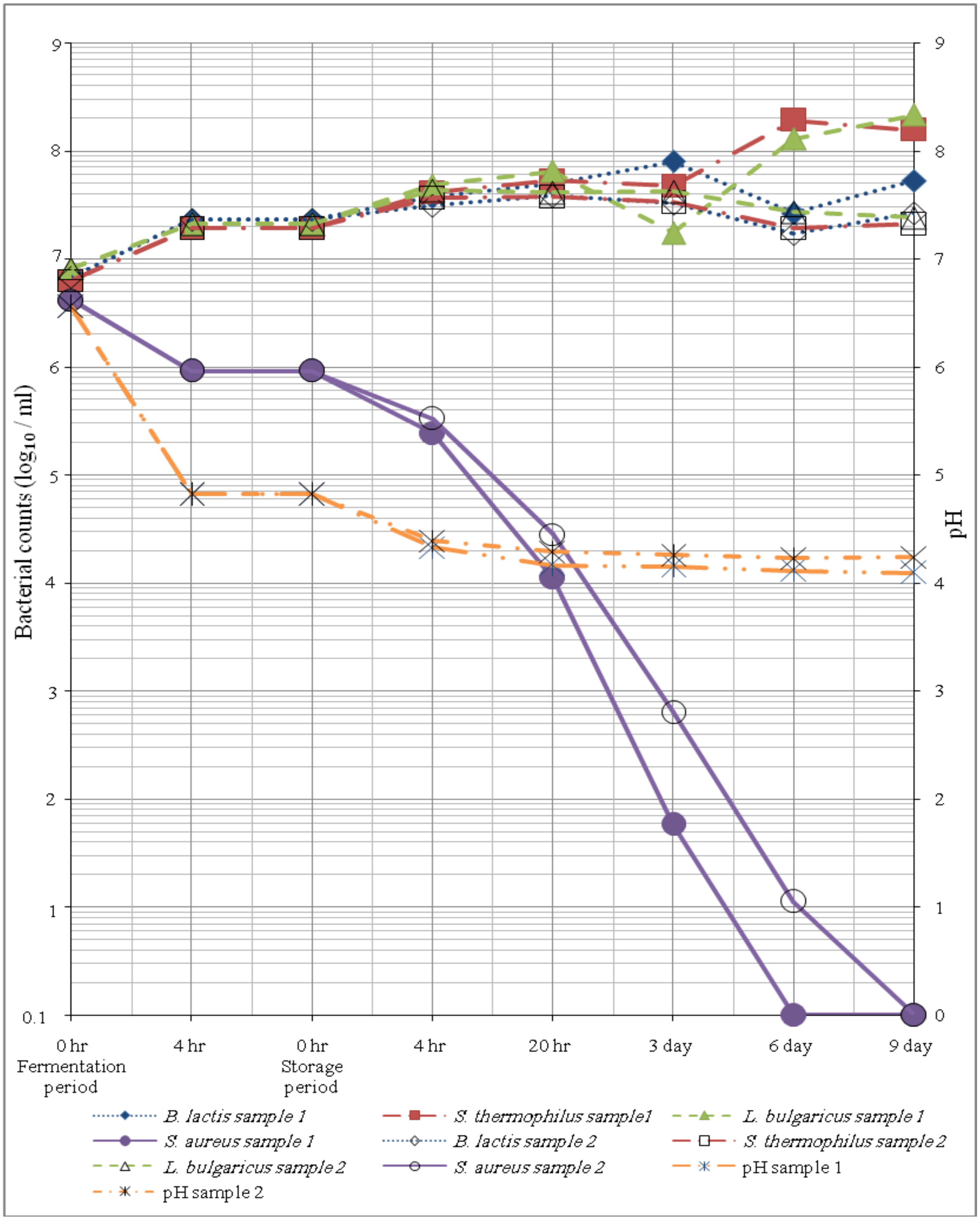


Figure 7: Growth interaction (log_{10} / ml) between yoghurt LAB starter culture, *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk after 4 hour incubation at 45° C. Stored in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during 9 days.

Figure 8 summarizes the growth inhibition interaction (inhibition percentage) of *S. aureus* (14) by *B. lactis*, yoghurt LAB starter culture (*S. thermophilus* and *L. bugaricus*) and the combination of both *B. lactis* and yoghurt LAB starter culture compared to growth of *S. aureus* (14) alone in fresh pasteurized cow's milk during 12 days of storage at room and refrigerator temperatures after an incubation period of 4 hour at 45° C. The results show that the highest inhibition of *S. aureus* (14) was by the combination of yoghurt LAB starter culture with *B. lactis*, followed by *B. lactis* alone and the least inhibition was by yoghurt LAB starter culture (Appendix C, Table 4).

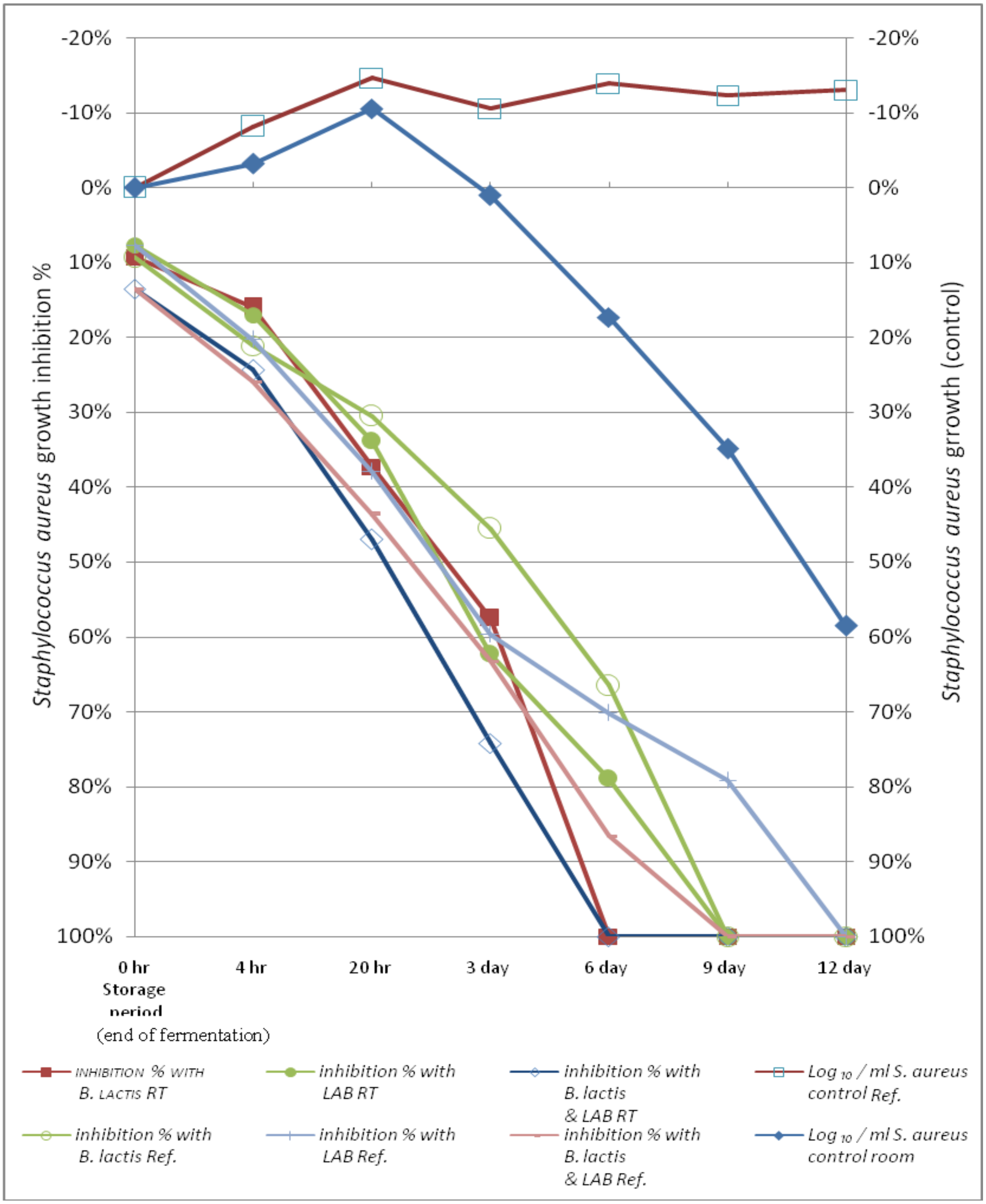


Figure 8: Growth inhibition % of *S. aureus* by *B. lactis*, yoghurt LAB starter culture and both *B. lactis* with yoghurt LAB starter culture compared to its growth alone in fresh pasteurized cow's milk during 12 days of storage at room (RT) and refrigerator (Ref.) temperatures, after 4 hour incubation at 45° C.

5. Conclusions and Recommendations:

5.1.: Conclusions:

The conclusions of this study show that:

Yoghurt LAB starter culture alone significantly controlled and completely inhibited the growth of *S. aureus* (14) in milk during fermentation and storage at room and refrigeration temperatures.

Similarly *B. lactis* alone significantly controlled and completely inhibited the growth of *S. aureus* (14) in milk during fermentation and storage at room and refrigeration temperatures.

Both of yoghurt LAB starter culture and *B. lactis* together significantly controlled and completely inhibited the growth of *S. aureus* (14) in milk during fermentation and storage, and more than the effect of any of yoghurt LAB starter culture or *B. lactis* alone.

The pH values of milk in general consistently decreased in all experiments during fermentation and storage

5.2.: Recommendations:

Further research to determine the interactions and mode of actions of LAB with *B. lactis* and other probiotic bacteria against *S. aureus* and other pathogens dairy products or other processed foods are needed.

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Appendix A:

Bifidobacterium lactis data sheet

Appendix B: culture media and chemical reagents

1. Baird parker agar (Oxoid):

Baird parker agar (BPA) has the following composition (g/L distilled water).

Trypton……………………………………….....10.0 g

Lab-lemco powder……………………………....5.0 g

Yeast extract……………………………………1.0 g

Sodium pyruvate………………………………10.0 g

Glycine…………………………….............12.0 g

Lithium chloride……………………………….....5.0 g

Agar…………………………………………20.0 g

2. Brain heart infusion broth (Oxoid):

Brain heart infusion (BHI) broth has the following composition (g/L distilled water).

Calf brain infusion solids…………………………12.5 g

Beef heart infusion solids………………………….5.0 g

Proteose peptone…………………………………10.0 g

Glucose…………………………………………….2.0 g

Sodium chloride……………………….................5.0 g

Di-sodium phosphate……………………………….2.5 g

3. DNASE agar (Oxoid):

DNASE agar has the following composition (g/L distilled water).

Tryptone…………………………………….20.0 g

Deoxyribonucleic acid………………………….2.0 g

Sodium chloride……………………………....5.0 g

Agar…………………………………………12.0 g

4. M17 agar (Himedia):

M17 agar has the following composition (g/L distilled water).

Soya peptone…………………………………5.0 g

Peptone…………………………………….5.0 g

Yeast extract…………………………………2.5 g

Beef extract………………………………….5.0 g

Lactose………………………………………5.0 g

Ascorbic acid……………………….............0.25 g

Magnesium sulphate………………….............10.0 g

5. de Man, Rogosa, and Sharpe agar (Oxoid) (Conda):

De Man, Rogosa, and Sharpe (MRS) agar has the following composition (g/L distilled water).

Peptone……………………………………10.0 g

Lab-lemco powder…………………….................8.0 g

Yeast extract…………………………………...4.0 g

Glucose……………………………………….20.0 g

Sorbitan mono-oleate………………...................1.0 ml

Di-potassium hydrogen phosphate………………..2.0 g

Sodium acitate $3H_2O$……………………………..5.0 g

Tri-ammonium citrate………………....................2.0 g

Magnesium sulphate $7H_2O$………………………0.2 g

Manganese sulphate $4H_2O$………………………0.05 g

Agar…………………………………………..10.0 g

6. Peptone water (Oxoid):

Peptone water has the following composition (g/L distilled water).

Peptone…………………………......................10.0 g

Sodium chloride………………………………..5.0 g

7. Tryptone soya agar (Oxoid):

Tryptone soya agar has the following composition (g/L distilled water).

Pancreatic digest of casein………………………17.0 g

Enzymatic digest of soya bean……………………3.0 g

Sodium chloride……………………………………5.0 g

Di-potassium hydrogen phosphate…………………2.5 g

Glucose………………………………………….2.5 g

Agar……………………………….....................10.0 g

8. Reagents and chemicals:

a. Acetic acid (J. T. Backer).

b. Anaerogen gas packs (Oxoid).

c. Di-sodium glycerophosphate (Hopkin & Williams) and (Sigma).

d. Egg yolk tellurite emulsion (Oxoid).

e. Hydrogen peroxide solution 3%.

f. HCL solution 1 N (Merck).

g. L- cysteine hydrochloride (Sigma).

h. Lithium chloride (Scharlab).

i. Rabbit plasma (Liofil-chem. s.r.i).

j. Sodium pyruvate (Seelze-Hannover).

k. Raffinose pentahydrate (Aldrich).

Appendix C

Table 1: Growth interaction (log / ml) between yoghurt LAB (*S. thermophilus* and *L. bulgaricus*) and *S. aureus* (14) in fresh pasteurized cow's milk in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during period of 6 days, after 4 hour incubation at 45° C.

Fermentation period at 45° C	Sample 1				Sample 2			
	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH
0 hr	7.4	7.57	7.6	6.55	7.4	7.57	7.6	6.55
4 hr	8.54	8.28	7.21	4.49	8.54	8.28	7.21	4.49
Storage period	Room temperature (20-25° C)				Refrigeration temperature (4° C)			
0 hr	8.54	8.28	7.21	4.49	8.54	8.28	7.21	4.49
4 hr	8.48	8.54	6.8	4.33	8.61	8.23	7.03	4.31
20 hr	8.69	8.75	5.97	4.28	8.53	8.34	6.32	4.26
3 day	9.3	9.17	3.97	4.13	8.14	8.43	4.57	4.23
6 day	9.12	9.25	1.83	4.01	8.22	8.36	3.11	4.18

Appendix C

Table 2: Growth interaction (log / ml) between *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during period of 6 days, after 4 hour incubation at 45° C.

Fermentation period at 45° C	Sample 1			Sample 2		
	Log_{10} / ml *B. lactis*	Log_{10} / ml *S. aureus*	pH	Log_{10} / ml *B. lactis*	Log_{10} / ml *S. aureus*	pH
0 hr	7.78	7.6	6.55	7.78	7.6	6.55
4 hr	8.49	6.97	5.65	8.49	6.97	5.65
Storage period	Room temperature (20-25° C)			Refrigeration temperature (4° C)		
0 hr	8.49	6.97	5.65	8.49	6.97	5.65
4 hr	8.9	6.25	5.21	8.6	6.31	5.62
20 hr	9.3	5	4.94	8.62	5.82	5.55
3 day	9.63	3.57	4.08	8.48	4.15	5.54
6 day	9.47	1.1	4.01	8.56	2.33	5.58

Appendix C

Table 3: Growth interaction (log / ml) between yoghurt LAB starter culture, *B. lactis* and *S. aureus* (14) in fresh pasteurized cow's milk in room (20-25° C, sample 1) and refrigeration (4° C, sample 2) temperatures and the pH values during period of 6 days, after 4 hour incubation at 45° C.

Fermentation period at 45° C	Sample 1					Sample 2				
	Log_{10} / ml *B. lactis*	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH	Log_{10} / ml *B.lactis*	Log_{10} / ml *S. therm-ophilus*	Log_{10} / ml *L. bulg-aricus*	Log_{10} / ml *S. aureus*	pH
0 hr	7.78	7.4	7.57	7.6	6.55	7.78	7.4	7.57	7.6	6.55
4 hr	8.4	8.34	8.08	6.72	4.41	8.4	8.34	8.08	6.72	4.41
Storage period	Room temperature (20-25° C)					Refrigeration temperature (4° C)				
0 hr	8.4	8.34	8.08	6.72	4.41	8.4	8.34	8.08	6.72	4.41
4 hr	8.65	8.29	8.17	6.16	4.25	8.37	8.41	8.15	6.02	4.29
20 hr	8.38	8.81	8.45	4.83	4.18	8.32	8.28	8.18	5.36	4.21
3 day	8.54	9.35	9.29	3.21	4.04	8.3	8.14	8.26	3.85	4.15
6 day	8.33	8.96	9.07	0.6	3.97	8.18	8.06	8.24	1.26	4.12

Appendix C

Table 4: Growth inhibition % of *S. aureus* (14) by *B. lactis*, yoghurt LAB starter culture and both *B. lactis* with yoghurt LAB starter culture compared to its growth alone in fresh pasteurized cow's milk during 12 days of storage at room and refrigeration temperatures, after 4 hour incubation at 45° C.

Fermentation period at 45° C	Sample 1							Sample 2						
	Log_{10} / ml *S. aureus* control	Log_{10} / ml *S. aureus* with *B. lactis*	inhibition %	Log_{10} / ml *S. aureus* with LAB	inhibition %	Log_{10} / ml *S. aureus* with *B. lactis* & LAB	inhibition %	Log_{10} / ml *S. aureus* control	Log_{10} / ml *S. aureus* with *B. lactis*	inhibition %	Log_{10} / ml *S. aureus* with LAB	inhibition %	Log_{10} / ml *S. aureus* with *B. lactis* & LAB	inhibition %
0 hr	6.78	6.62	NA	6.62	NA	6.62	NA	6.78	6.62	NA	6.62	NA	6.62	NA
4 hr	6.89	6.25	9.29%	6.36	7.69%	5.96	13.50%	6.89	6.25	9.29%	6.36	7.69%	5.96	13.50%
Storage period	Room storage (20-25° C)							Refrigeration storage (4°C)						
0 hr	6.89	6.25	9.29%	6.36	7.69%	5.96	13.50%	6.89	6.25	9.29%	6.36	7.69%	5.96	13.50%
4 hr	7.11	5.98	15.89%	5.9	17.02%	5.38	24.33%	7.45	5.88	21.07%	5.94	20.27%	5.52	25.91%
20 hr	7.62	4.78	37.27%	5.04	33.86%	4.04	46.98%	7.9	5.49	30.51%	4.9	37.97%	4.45	43.67%
3 day	6.82	2.9	57.48%	2.57	62.32%	1.76	74.19%	7.62	4.15	45.54%	3.07	59.71%	2.8	63.25%
6 day	5.7	0	100.00%	1.2	78.95%	0	100.00%	7.85	2.63	66.50%	2.34	70.19%	1.05	86.62%
9 day	4.49	0	100.00%	0	100.00%	0	100.00%	7.74	0	100.00%	1.61	79.20%	0	100.00%
12 day	2.86	0	100.00%	0	100.00%	0	100.00%	7.79	0	100.00%	0	100.00%	0	100.00%

تداخل نمو بادىء بكتيريا حامض اللاكتيك مع او بدون اضافة *Bifidobacterium spp.* على نمو *Staphylococcus aureus* خلال تخمر الحليب وتخزينه.

اعداد

ياسر محمد خليل حمامده

المشرف

الأستاذ الدكتور خلف صوفي الدليمي

ملخص

استهدفت هذه الدراسة معرفة تأثير النمو فيما بين البكتيريا المرضية العنقودية الذهبية *Staphylococcus aureus* وبكتيريا حامض اللاكتيك (بادىء تخمر الحليب الى اللبن) والبكتيريا النافعة *Bifidobacteium lactis* كل على حده ومجتمعة اثناء فترة التخمر (لمدة 4 ساعات على درجة حرارة 45° مئوية) والخزن بعد ذلك على درجتي حرارة الغرفة(20-25° م) والتبريد (4° م) لمدة 12 يوما. لقد اظهرت النتائج ان تنمية البكتيريا العنقودية الذهبية مع بكتيريا البادىء ادى الى انخفاض معنوي (على $P < 0.01$) في العدد الابتدائي للبكتيريا العنقودية الذهبية (لوغاريثم / مل) اثناء فترة التخمر والخزن (لوغاريثم 1.2 بعد 6 ايام و الى الصفر بعد 9 ايام و لوغاريثم 1.61 بعد تسعة ايام والى الصفر بعد 12 يوم) في كل من درجتي حرارة الغرفة والتبريد على التوالي. وقد ازداد عدد بكتيريا البادىء خلال فترة الخزن في درجة حرارة الغرفة. لقد لوحظ ان انخفاض عدد البكتيريا العنقودية الذهبية وازدياد عدد بكتيريا البادىء اقل نسبيا خلال التبريد منه خلال الخزن في درجة حرارة الغرفة. كما وجد ان نتيجة تنمية البكتيريا العنقودية الذهبية مع البكتيريا النافعة *Bifidobacterium lactis* كانت بصورة عامة متماثلة مع نموها مع بكتيريا البادىء. حيث انخفض عدد البكتيريا العنقودية الذهبية الى لوغاريثم 2.9 بعد 3 ايام والى الصفر بعد 6 ايام بدرجة حرارة الغرفة والى لوغاريثم 2.63 بعد 6 ايام والى الصفر بعد 9 ايام بالتبريد. وعند تنمية البكتيريا العنقودية الذهبية مع خليط من بكتيريا البادىء والبكتيريا النافعة *Bifidobacterium lactis* وجد ان التاثير المضاد على نموها اعلى من اي منهما على انفراد (حيث انخفض عدد البكتيريا العنقودية الذهبية الى لوغاريثم 1.76 بعد ثلاثة ايام والى الصفر بعد 6 ايام في درجة حرارة الغرفة والى لوغاريثم 1.05 بعد 6 ايام والى الصفر بعد 9 ايام في درجة حرارة التبريد). عند زيادة اعداد البكتيريا تحت التجربة لكل منها عشرة اضعاف (لوغاريثم واحد) لم يؤدي ذلك الى تغير معنوي في اعدادها. لقد وجد ان قيم الاس الهيدروجيني (pH) انخفض انخفاضا معنويا على ($P < 0.01$) خلال فترتي التخمر والخزن.

و يستخلص من هذه الدراسة ان البكتيريا المرضية العنقودية الذهبية اضمحلت الى الصفر نتيجة نمو بكتيريا البادىء والبكتيريا النافعة *Bifidobacterium lactis* في درجتي حرارة الغرفة والتبريد.

ولذلك من الممكن ان يوصى باستخدام البكتيريا النافعة *Bifidobacterium lactis* مع بكتيريا بادىء تخمر الحليب الى اللبن خلال فترة التخمر والخزن.